Background Document on Health Effects Studies and Prevalence Estimates Used in the Preliminary Initial Regulatory Flexibility Analysis (PIRFA) For Occupational Exposure to Flavorings Containing Diacetyl

This document provides background information for two sections of the PIRFA. Part A is an expanded discussion of the health effects studies that examine respiratory disease among employees exposed to diacetyl as well as flavorings containing diacetyl and a number of other constituent chemicals. Part B explains, in detail, the prevalence estimates of airway obstruction used to calculate the projected benefits that would result from implementing a draft standard.

PART A

Health Effects Studies

A number of studies have described adverse health effects among employees exposed to diacetyl and flavorings containing diacetyl, including several occupational investigations and case reports that have documented obstructive airway disease among employees exposed to airborne butter flavoring chemicals (Kanwal, 2008). While a few earlier investigations uncovered obstructive lung disease among employees at flavoring manufacturing sites, the scientific community did not become aware of flavoring-related obstructive airway disease until 2000 after a case cluster was identified at a microwave popcorn production plant. Subsequent investigations at microwave popcorn production plants demonstrated higher-than-expected rates of respiratory symptoms such as chronic cough, shortness of breath, and wheezing among employees. In some cases, these effects may be symptomatic of a potentially disabling obstructive lung disease known as constrictive bronchiolitis obliterans. Higher-than-expected rates of physician-diagnosed asthma and chronic bronchitis have also been reported. Employees exposed to butter flavoring also experience eye, skin, nose, and throat irritation.

As indicated above, spirometry surveys in the investigations of microwave popcorn production plants revealed higher-than-expected prevalences of *airway*

obstruction, defined as a reduction in FEV_1 and FEV_1/FVC ratio[1] among employees exposed to the butter flavoring chemicals. Airway obstruction is described as *fixed* when abnormal pulmonary function test results does not improve with bronchodilator treatment. Severe airway obstruction refers to FEV_1 decrements that are lower than 40 to 50 percent of normal. The onset of flavoring-related obstructive airway disease has been reported to occur over a few months to several years (Akpinar-Elci et al., 2004).

Fixed airway obstruction is characteristic of bronchiolitis obliterans. This lung disease results from inflammation and scarring of the tissue lining the small airways of the lung. In response to the damage, the airways become thickened, narrowed, and sometimes completely obstructed limiting movement of air into and out of the lung. Because it is an uncommon condition, bronchiolitis obliterans may be misdiagnosed as the more frequently encountered obstructive lung diseases of chronic bronchitis, emphysema, or asthma. However, unlike chronic bronchitis and emphysema which frequently occur in older individuals with a history of heavy smoking, bronchiolitis obliterans is often found among non-smoking individuals under 50 years of age. A high resolution computerized tomography (CT) scan or, sometimes, a specialized lung biopsy is needed to confirm a diagnosis of bronchiolitis obliterans. As airways become more severely damaged, employees with bronchiolitis oliterans suffer persistent symptoms and permanent loss of pulmonary function. Several employees with severe disease are on waiting lists to receive lung transplants. At least three employees with flavoring-related bronchiolitis obliterans have died (Egilman et al., 2007).

Several sources of data have contributed to the recognition of flavoring-related obstructive airway disease in the workplace and the identification of potential risk factors. NIOSH conducted medical and industrial hygiene evaluations at six microwave popcorn production plants after a cluster of disease was uncovered in a Missouri plant. NIOSH also investigated lung disease at three food flavor manufacturing facilities. The National Jewish Medical Research Center has reported preliminary results from medical surveillance and exposure monitoring of workers at eleven other food flavor

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1. The most common pulmonary function tests, including FEV_1 and FVC, are often measured using *spirometry*, which measures the flow of air in and out of the lungs. FEV_1 is the volume of air that a peson can exhale through a mouthpiece in one second. Forced vital capacity (FVC) is the amount of air that can be exhaled following full inspiration. For accurate measurement of FVC, a person must inhale as deeply as possible and then exhale as forcefully as possible through a mouthpiece, for as long as possible.

manufacturing sites. Eight of these plants are also part of the Flavor Industry Safety and Health Evaluation Program (FISHEP), a state cooperative compliance initiative in California involving 28 flavor manufacturing establishments. The Netherlands Expertise Centre for Occupational Respiratory Disorders evaluated the prevalence of bronchiolitis obliterans among employees at a diacetyl production plant who were potentially exposed to the chemical. Diacetyl is a major volatile chemical component of artificial butter flavoring and the primary compound responsible for the buttery odor and taste. Finally, there have been preliminary inhalation studies in rodents exposed to diacetyl and vapors of a butter flavoring mixture.

Investigations of Microwave Popcorn Plants

The hazards associated with butter flavoring came under scrutiny with the diagnosis of bronchiolitis obliterans in eight former employees who had worked in mixing and packaging operations at a Missouri microwave popcorn plant (Parmet, 2002). A ninth case from the same plant was discovered shortly afterwards (Akipinar-Elci et al., 2004). The individuals ranged in age from 27 to 51 years of age and had worked at the plant from five months to nine years (median, 1.5 years) before onset of symptoms. Six of the injured workers were current or former smokers. All cases were found to have severe airway obstruction (i.e., range of FEV_1 from 15 to 40 percent of predicted) and characteristic symptoms such as cough, and progressive exertional dyspnea. Eight cases had tissue imagery on high resolution CT consistent with bronchiolitis obliterans and two had findings on thoracoscopic lung biopsy consistent with . Five cases were placed on a lung transplant candidate list. One case died before receiving a lung transplant.

Five of the former employees had worked in the mixing room where butter flavorings and oil are mixed. The other four employees had worked on the packaging lines where popcorn kernels and the oil/flavorings mixture are added to microwaveable bags and packaged for shipment. In this plant, the packaging lines were located in close proximity to the mixing area. These cases were from a pool of roughly 300 production workers employed at the Missouri plant between 1992 and 2000 (MMWR, 2002). As a comparison, the estimated prevalence of severe airway obstruction (i.e., $FEV_1 < 40$ percent) among the non-smoking US population under 50 years of age is about 0.1

percent (one case per 1000 individuals).

NIOSH evaluated the medical condition of 117 employees currently working at the Missouri plant in November 2000 (Kreiss et al., 2002). Using a medical questionnaire and pulmonary function testing, NIOSH found that 23 percent (19 individuals) of employees that worked in production, quality control and maintenance had fixed airway obstruction (i.e. both FEV_1, FEV_1/FVC below the lower limit of normal and not improved with bronchodilator treatment). Three of these cases had severe airway obstruction. The prevalence of airway obstruction was 3.3 times higher than expected for all employees and 10.8 times higher than expected for employees who had never smoked based on national statistics. Medical symptoms that were elevated among production workers included chronic cough, exertional dyspnea (i.e., shortness of breath upon exertion), wheezing, physician diagnosed asthma and chronic bronchitis, unusual fatigue, skin and mucous membrane irritation. Rates of physician-diagnosed asthma and chronic bronchitis were also higher than expected.

NIOSH conducted area air monitoring at the Missouri plant for volatile organic chemicals (VOCs) and respirable dust in September 2000 (Kullman et al., 2005). There were more than 100 VOCs identified in the plant on initial sampling. Several of the most prominent airborne VOCs, such as diacetyl, acetoin, 2-nonanone, acetic acid, and butyric acid were constituents of the butter flavoring. NIOSH reported that the average air levels of diacetyl in the mixing room, microwave popcorn packaging lines, quality control area, and non-production areas were 37.8 ppm (range: 2.3-98 ppm), 1.7 ppm (0.26-5.5 ppm), 0.54 ppm (0.33-0.89 ppm) and 0.04 ppm ($\leq$0.25 ppm) respectively[2]. Using the diacetyl air measurements as an exposure marker for butter flavoring vapor concentrations, NIOSH observed a statistically significant increasing trend between the proportion of employees with airway obstruction and quartiles of cumulative exposure (i.e., average air level in area worked multiplied by the number of years worked in area).

Employees with the highest cumulative exposures were almost exclusively mixers who poured the open containers of butter flavoring mixes into a large improperly sealed

[2] NIOSH subsequently determined that the diacetyl sampling method used in these investigations can be affected by relative humidity and that high humidity levels may result in an underestimation of true airborne diacetyl concentrations (NMAM, 2008). NIOSH is working to develop a set of correction factors and to validate a new method for the measurement of diacetyl in the workplace.

mixing tank containing heated soybean oil and nearby workers who operated the packaging machines where the butter flavored oil was combined with kernel popcorn in a microwavable bag. NIOSH noted that five of six employees who worked in the quality control room popping nearly 100 bags of product in microwave ovens per shift suffered airway obstruction despite relatively low full shift exposure to butter flavoring vapors (e.g., 0.5 ppm diacetyl). Task-based air sampling, however, revealed 0.75 to 4.4 ppm diacetyl in the exhaust area during popping and peak blasts as high as 13 ppm diacetyl while pouring freshly popped corn from bags into containers. This result suggests that regular brief periods of high exposures might contribute to the risk of airway obstruction.

NIOSH continued to regularly conduct air monitoring and spirometry of employees of the Missouri plant into 2003, even as engineering controls were implemented to reduce worker exposures (NIOSH, 1/2006). By the summer of 2003, average diacetyl levels were reduced to 0.4 ppm in the mixing area and to below 0.1 ppm in the packaging and quality control areas. The decline in mean annualized FEV_1 among plant employees over time had leveled off somewhat by 2003 (Kreiss, 2007). Mixers hired after improvements in mixing room ventilation suffered less pulmonary function loss over the post-implementation period than mixers hired prior to the improvements in ventilation. NIOSH concluded from its investigations of the Missouri microwave popcorn plant that the high rates of airway obstruction and occupational bronchiolitis obliterans initially found among the production employees were probably caused by inhalation of excessive amounts of volatile butter flavoring ingredients.

NIOSH received requests to investigate five other microwave popcorn plants in the Midwest between 2001 and 2003 (NIOSH, 5/2003, 7/2003, 10/2003, 7/2004, 12/2004). The plants varied greatly in size and operation. The smallest had only six employees, one mixing tank and one packaging line. The largest had a workforce of 313 with more than five mixing tanks and more than seven packaging lines. Two of the plants had all or some of their tanks of heated oil and butter flavorings located adjacent to packaging lines similar to the Missouri plant. The other three plants had isolated the packaging area from the mixing tanks prior to NIOSH investigation. As an indicator of exposure to butter flavoring vapors, full shift area and personal time-weighted average (TWA) air concentrations of diacetyl were measured in several job locations of all plants.

In some cases, NIOSH was able to measure diacetyl, in the breathing zone of mixers while performing high exposure tasks such as pouring containers of butter flavoring into open tanks of heated oil. A medical survey consisting of a review of medical records, a respiratory symptom questionnaire and lung spirometry was conducted on current employees at each plant.

The average full shift air levels of diacetyl in the mixing areas of the five plants ranged from 0.2 to 1.2 ppm which was considerably less than the readings at the Missouri plant prior to intervention (Kanwal et al., 2006). Several task-based measurements at one plant showed that the diacetyl concentrations averaged 5 to 10 ppm for 30 to 60 minutes following the open transfer of butter flavoring to heated mixing tanks (NIOSH, 12/2004). At another plant, real-time monitoring revealed peak diacetyl air concentrations of over 80 ppm for several minutes in the breathing zone during an open transfer operation (NIOSH 7/2004). In the two plants where packaging and butter flavoring mixing activities were in close proximity, average full shift diacetyl levels were 0.4 to 0.6 ppm. On the other hand, average diacetyl concentrations were 0.03 ppm or less in the packaging areas of the three plants that were isolated from the mixing tanks. Review of medical records showed that six employees, three mixers and three packaging line operators, from four of the five microwave popcorn plants had fixed airway obstruction and CT scans consistent with bronchiolitis obliterans. NIOSH aggregated the medical survey data for 537 employees from the six investigated microwave popcorn plants for production job categories of mixers, packaging area workers, quality control, and maintenance (Kanwal et al., 2006). Employees that worked as mixers generally had higher prevalence of chronic cough, dyspnea, and wheezing and lower mean percent predicted FEV_1 than employees without mixing experience. This was particularly evident among mixers with more than 12 months experience and who never smoked. The prevalence of fixed airway obstruction among this group was 15 to 20 percent. The prevalence of fixed airway obstruction among packaging workers without mixing experience in the plants where packaging lines were not isolated from the mixing tanks was 14 percent as opposed to 5.5 percent of packaging workers in plants where the packaging area was isolated from the mixing tanks.

The NIOSH investigations show that the flavoring-related obstructive airway

disease initially uncovered among production employees at the Missouri plant also occurred at other microwave popcorn plants. The risk of airway obstruction and respiratory symptoms among employees correlates with exposure to butter flavoring vapors. Disease prevalence was greatest among mixers and packaging line operators at the Missouri plant where air levels of diacetyl and other volatile butter flavoring chemicals were the highest. The mixers and packaging operators who worked in close proximity to the mixing operations at the other plants were exposed to lower levels of butter flavoring vapors and experienced a somewhat smaller but still elevated prevalence of airway obstruction and respiratory symptoms. The lowest rates of airway disease at the plants were experienced by the production and non-production employees with the least exposure. NIOSH concluded that microwave popcorn workers at plants like these were at risk of flavoring-related lung disease and that respiratory protection and engineering controls were necessary to protect workers.

Although not of occupational origin, a case of bronchiolitis obliterans in affected microwave popcorn workers was identified in a consumer who had daily inhalation exposure to butter flavoring compounds from heavy daily consumption of microwave popcorn (Rose, 7/2007). The case had no known other occupational or environmental exposures that would explain his disease. Airborne measurements of diacetyl for short periods during microwave popcorn preparation in the patient's home were reported to be similar to levels measured in the microwave oven area during corn popping in the quality assurance unit of the Missouri plant.

Investigations of Food Flavor Manufacturing Plants

Cases of flavoring-related airway disease have also been reported and investigated is food flavor manufacturing. Prior to the discovery of the cluster of bronchiolitis obliterans cases in the Missouri microwave popcorn plant, there were two earlier reports that identified obstructive airway disease in flavor manufacturing. NIOSH described severe fixed airway obstruction compatible with bronchiolitis obliterans in two former employees of a company that blended large batches of flavoring ingredients with corn starch and flour to make 'cinnabutter' and other flavors for use in the baking industry (NIOSH, 7/1986). Both mixers were non-smokers under 31 years of age. They reported

dyspnea and persistent cough within six months of employment. Two other plant employees who had previously worked in the mixing room were asymptomatic but developed mild airway obstruction. Both workers were ex-smokers under 40 years of age. NIOSH measured dust levels at the plant but did not sample for volatile chemicals. Researchers at the University of Cincinnati College of Medicine reported severe respiratory disease with clinical findings consistent with bronchiolitis obliterans in five employees at a large flavor manufacturing facility (Lockey et al., 2002). The index case was discovered in 1996 and subsequent cases were uncovered as part of regular workplace spirometry screening. It was noted that the employees were exposed to acetaldehyde and a large number of other flavoring agents that had not been studied for inhalation toxicology. One of those agents was later determined to be diacetyl.

Once the findings from the Missouri microwave popcorn cluster became known, the food flavor manufacturing sector began to receive more scrutiny. The National Jewish Medical and Research Center (NJMRC) conducted industrial hygiene surveys and medical surveillance of flavor manufacturing facilities beginning in 2002. NJMRC characterized breathing zone exposures to diacetyl, and other high priority flavoring chemicals during production processes that used the highest amounts of these substances. They collected full-shift and task-based air samples. Diacetyl exposures were also characterized in quality control and non-production areas. Medical surveillance focused on medical and occupational history, respiratory symptoms and spirometry. Spirometry was not repeated after bronchodilator administration.

NJMRC recently aggregated and reported preliminary results of investigations of eleven flavor manufacturing plants most of which had fewer than 100 total employees (Rose et al., 2007). Some plants specialized in a particular type of flavor (e.g. butter and cream) while others produced many different types flavorings and fragrances. Most facilities had areas for liquid and powder compounding (production processes), quality control laboratories, warehouses for shipping and storage, and administrative offices. Medical surveillance was done on 371 employees of which about half worked in production. The production employees reported higher than expected rates of respiratory symptoms and asthma. The study also found that employees with the highest cumulative

exposures to diacetyl were more likely to experience process-related breathing problems and eye, nose, and throat irritation than employees with the lowest cumulative exposures.

The average production process-associated personal diacetyl air level at the NJMRC-investigated flavor manufacturing facilities was 2.5 ppm (Martyny et al., 2008). Production process-associated air levels were measured over an approximately one to two hour period needed to produce liquid or powder formulations using the NIOSH sampling method. They were regarded as exposure levels that result from high-end rather than routine production operations. The production process-associated diacetyl levels were generally higher for powder than liquid compounding. The frequency with which diacetyl- containing flavors were manufactured ranged from daily to once a year among the plants. Mean full-shift TWA diacetyl exposures in quality control, warehousing, and administrative areas were substantially less than production. Other measured chemicals with notable air concentrations during flavor production were acetoin, acetic acid, acetaldehyde, and benzaldehyde.

The State of California began an active investigation of obstructive airway disease among flavoring manufacturing establishments in the State after learning of two employees with confirmed bronchiolitis obliterans at separate Southern California plants. By January 2007, six additional employees with suspected fixed obstructive lung disease had been identified (Materna, 2007). The eight individuals were flavoring compounders employed at five Southern California flavor manufacturing facilities. Their jobs involved mixing chemicals, including diacetyl, to make food flavorings. All cases were under 50 years of age with no known smoking history. They first experienced characteristic symptoms (e.g., persistent cough, wheezing and shortness of breath) from one month to five years from start of employment and these became progressively worse despite treatment for asthma, allergic rhinitis, or chronic bronchitis. Spirometry indicated that the cases had severe airway obstruction with FEV_1 measurements ranging from 18 to 45 percent of predicted based on age, gender, height and weight.

In April 2006, Cal/OSHA announced FISHEP, a cooperative compliance effort to assess the respiratory health and exposure to potentially hazardous chemicals among flavor manufacturing employees. Twenty-eight food flavoring manufacturing facilities in the State of California are enrolled in FISHEP. The assessments involve pulmonary

function testing of all plant employees and air monitoring for diacetyl and other flavoring chemicals. These are primarily performed by qualified non-government consultants and the results are made available to California authorities and the manufacturing company. The companies agree to implement any recommended steps needed to reduce risk of respiratory disease from exposure to flavoring substances.

FISHEP collected initial pulmonary function testing on 474 workers (Cal/OSHA, 2007). Twenty-six employees from nine different flavor manufacturing facilities were found to have abnormal spirometry. The abnormalities were considered mild for all but three individuals. Further testing will determine whether the abnormal lung spirometry is fixed airway obstruction. Preliminary exposure monitoring in eleven of the facilities show that mean full shift time-weighted average personal diacetyl exposure among production employees ranges from less than 0.08 to 1.7 ppm.

NIOSH was asked to conduct health hazard evaluations of two Southern California flavor manufacturing plants (NIOSH, 3/2007; 4/2007). The plants were similar in terms of size, operation, and production volume. The combined workforce consisted of 83 current employees. Approximately 30 of these had worked in the production area manufacturing liquid and powder flavor products, including butter and cream flavors containing diacetyl. Medical surveys revealed four current or former production employees with severe fixed airway obstruction at the two plants. There was one current employee with mild obstruction. All impaired employees worked in powder production and handled diacetyl or diacetyl-containing flavors.

Extensive air sampling was completed at one plant and is still being collected and compiled at the other. The powder production area had a mean and median full-shift TWA diacetyl air levels in the plant of 0.25 ppm (range: 0.002 ppm to 1.1 ppm) and 0.06 ppm, respectively (NIOSH 4/2007). Unlike the NJRMC investigation that monitored the highest diacetyl exposure operations, NIOSH measured full-shift exposures during a week of routine operations that did not always include production of butter flavorings. However, NIOSH did measure mean production process-associated TWA diacetyl air levels of 7.7 to 21 ppm during one to two hour productions of diacetyl-containing butter-flavored and vanilla-flavored powders. Real time diacetyl peak levels between 20 and 200 ppm were measured in the operator breathing zone during short-term tasks of

pouring liquid diacetyl into the blender, filling containers with flavored powder, and cleaning the blender. Mean full-shift TWA air concentrations of diacetyl among employees in the liquid production area, laboratory, warehouse, and administrative offices were 0.03 ppm or less. Other flavor compounds with notable full shift TWA air concentrations (e.g. >0.1 ppm) in the production areas were acetoin, benzaldehyde, acetaldehyde, and acetic acid.

The pattern of evidence that indicates a risk of flavoring-related obstructive airway disease among food flavor manufacturing is strikingly similar to that of microwave popcorn production. Several cases of bronchiolitis obliterans and severe airway obstruction were uncovered among employees who regularly handled and blended diacetyl and other butter flavoring ingredients during production of the flavor product. Like microwave popcorn, the prevalence of self-reported respiratory symptoms and airway obstruction measured by spirometry were elevated in production employees with the highest cumulative exposures. Workplace air levels of the volatile butter flavoring marker chemical, diacetyl reported in powder production areas of flavor manufacturing plants were comparable to those found in the mixing areas of microwave popcorn plants. One important distinction is that employees who work in mixing operations at microwave popcorn plants are typically exposed to butter flavorings on a daily basis while exposure occurs less frequently at some flavor manufacturing facilities.

Production of Diacetyl and Foods that Use Diacetyl-Containing Flavors Other than Microwave Popcorn

In addition to microwave popcorn production and food flavoring manufacturing, cases of bronchiolitis obliterans were reported at a chemical plant that produced diacetyl in the Netherlands (Van Rooy et al., 2007). The Netherlands Expertise Centre for Occupational Respiratory Disorders and the Institute of Risk Sciences at Utrecht University investigated respiratory disease among 175 current and former employees at the plant who were potentially exposed to diacetyl from 1960 to 2003. The production process also yielded acetoin as a co-product and acetaldehyde and acetic acid as by-products. The employees with the highest diacetyl exposure potential were the 102 employees who worked as process operators. The study population was evaluated by a

standardized medical questionnaire and pulmonary function testing. Fixed airway obstruction was defined as $FEV_1/FVC \leq 70$ percent and $FEV_1 < 80$ percent of predicted which was not improved with bronchodilator treatment. A bronchiolitis obliterans case was defined by fixed airway obstruction combined with high resolution CT chest imagery typical of the disease. The plant collected a limited number of process- and task-based diacetyl area and personal samples between 1995 and 2003.

Four employees in the study, all of whom were diacetyl process operators, had fixed airway obstruction. Three of these individuals had CT scans consistent with bronchiolitis obliterans and severe obstruction with FEV_1 ranging from 35 to 42 percent of predicted. The time between first employment and onset of symptoms for two of these employees was one year or less. The third case reported onset of symptoms 14 years after the start of employment at the diacetyl production plant. The fourth individual had mild obstruction and reported no symptoms. After completion of the study, an additional case of severe fixed airway obstruction consistent with bronchiolitis obliterans was uncovered in a non-participating plant process operator. All impaired diacetyl process operators had co-exposures to acetoin and acetaldehyde as well as respiratory tract irritants (e.g. chlorine, ammonia) from other work environments within the plant.

The prevalence of certain symptoms, such as trouble breathing, daily cough, and physician-diagnosed asthma were significantly higher ($p<0.05$) among the study population than the general Dutch population when adjusted for smoking, age, and standing height (Van Rooy et al., 2008). While lung function did not differ between the potentially exposed diacetyl workers and the general population sample, multiple linear regression analysis found that FEV_1 values for process operators were significantly lower ($p<0.05$) than an internal reference group of minimally exposed workers. A relationship between abnormal pulmonary function and estimated cumulative diacetyl exposure could not be established. The authors stated that an exposure – response relationship in the study may have been obscured by misclassification as a result of considerable uncertainty in reconstructing cumulative exposures from the limited measurement data.

Acetoin and diacetyl were produced by oxidation of 2.3-butylene glycol in an enclosed reactor vessel at around 360 degrees Centigrade. These reaction products were then further separated and purified in a series of distillations and condensations. Process

operators were not exposed to the heated reactor products but may have been exposed during subsequent processing, particularly while transferring final product into containers. The geometric mean diacetyl levels measured in area samples during the diacetyl production process was 2.3 ppm. The geometric mean personal diacetyl exposure measured during the approximately one hour task of tapping 50 kg containers of liquid diacetyl was 11 ppm. Diacetyl was not analyzed using the NIOSH sampling method. Air levels of acetoin were not monitored at the plant. The frequency with which process operators worked in diacetyl production operations was stated to vary over time and among different workers. The authors concluded that their findings supported the hypothesis that the diacetyl production process caused bronchiolitis obliterans in exposed operators but that the precise causal agent responsible could not be established from their study. Nevertheless, the reduced number of chemical agents present in diacetyl production compared to the much higher number of chemical ingredients found in butter flavoring vapor might assist in prioritizing the list of flavoring compounds that need to be further scrutinized.

As yet, there has not been an organized study of occupational exposure and respiratory disease associated with the use of butter and other diacetyl-containing flavors in food production other than microwave popcorn. Butter flavoring is used in a variety of snack foods, cooking oils, dairy products, candies, cakes, and cookies. There have been anecdotal reports of lung disease in food production employees that are suspected to involve inhalation of flavorings (Kreiss, 2007). NIOSH has recently been requested to conduct a systematic evaluation of where diacetyl-containing food flavorings are being used, industries and operations where workers are exposed to diacetyl-containing food flavorings and what health effects workers might be experiencing from those exposures (U.S. House of Representatives, 1/2008). They are planning to conduct an industry wide research study to evaluated exposure to flavorings in the flavorings and food industries (73FR12179-12180, March 6, 2008)

Inhalation Studies in Experimental Animals

Respiratory tract toxicity has been investigated in rats and mice that inhaled butter flavoring vapors and pure diacetyl. The studies varied widely in terms of dose levels and number of exposures as described below.

An acute inhalation study was done in male and female Wistar rats using a standard study design to determine the concentration of diacetyl vapor that killed 50 percent of the animals (LC_{50}). No mortality occurred at the low concentration of 2.25 mg/l (640 ppm) but all animals died at 5.2 mg/l (1479 ppm) and 23.9 mg/l (6800 ppm) following a single four hour exposure (BASF, 1993). The LC_{50} was between 2.25 mg/l and 5.2 mg/l. The rats that died from exposure to the highest dose levels had hyperemia of the lungs, bronchial edema, and moderate emphysema. There was no pathology noted in the rats exposed to the low dose level.

NIOSH examined the effects of liquid butter flavoring vapors (BFV) and pure diacetyl on the respiratory tract of rats exposed to a one-time six hour inhalation study (Hubbs et al., 2002; 2008). Groups of male Sprague Dawley rats were exposed to low, medium and high vapor concentrations of BFV or pure diacetyl. The mean diacetyl concentrations in the low, medium, and high BFV were 202, 285, and 371 ppm, respectively. Respiratory tract tissue was examined by histopathology and by electron microscopy one day after exposure. All rats exposed to the medium and high BFV concentrations had moderate to severe multifocal necrotizing bronchitis which was most consistently present in the main bronchus (Hubbs et al., 2002). Transmission electron microscopy revealed that the site of the necrosis was the bronchial epithelium with the injury reaching beneath the basement membrane. The group of rats that received the high BFV concentration in a pulsed (i.e., BFV administered in a bolus at 0 and at 3 h) rather than constant (i.e., BFV administered at a steady rate for 6h) exposure suffered damage that extended beyond the main bronchus into the midsize bronchioles. No bronchial pathology was evident in control rats or rats exposed to the low concentrations of BFV. The alveoli of the lung were unaffected at all BFV concentrations. Suppurative inflammation and necrosis of the nasal tissue was observed at all concentrations of BFV tested. In addition to diacetyl, the vapors contained a complex mixture of several compounds including other ketones (e.g. acetoin and 2-nonanone) and organic acids (e.g. acetic acid and butyric acid).

The rats exposed to pure diacetyl showed epithelial injury similar to BFV-exposed animals but covered a less extensive area of the respiratory tract (Hubbs et al., 2008). Diacetyl inhalation caused dose-dependant inflammation and necrosis of the nasal epithelium at 122 ppm and higher. At 225 ppm and higher, diacetyl-induced necrosis extended to the level of the trachea and larynx. The mainstem bronchii were affected at 295 ppm and higher. There was no difference in respiratory damage whether the total diacetyl dose was administered continuously over six hours or in four 15 minute pulses. There was no significant damage to the upper respiratory tract in rats inhaling 99 ppm continuously over six hours but a similar dose administered in a single 1800 ppm pulse did produce signs of damage in the nose. There was no significant pathological changes in bronchiolar epithelium or alveoli at any diacetyl concentration.

The National Institute of Environmental Health Sciences (NIEHS) evaluated the respiratory tract toxicity in C57BL/6 mice exposed to repeated inhalations of pure diacetyl for up to twelve weeks (Morgan et al., 2008). In the subacute phase of the study, groups of mice were exposed to 200 and 400 ppm diacetyl, six hours per day for five days. These exposures caused some deaths and a dose-dependent necrotizing rhinitis, laryngitis, and bronchitis to the level of the proximal large bronchi but no bronchiolar or lung lesions. In an attempt to reduce nasal tissue toxicity, daily inhalation exposures to 100, 200, and 400 ppm diacetyl were decreased to 1 hour per day, five days a week and the exposure period extended to four weeks. Mild to moderate dose-dependent chronic bronchitis, laryngitis, and rhinitis were present at all dose levels at two and four weeks. There was no tissue ulceration and necrosis at 200 ppm or below. Most mice had minimal peribronchiolar lymphocytic imflammation after a four week exposure to 400 ppm. A group of mice were subjected to a pulsed diacetyl exposure of 1200 ppm for 15 minutes, twice a day, five days a week for four weeks. This exposure regimen produced a greater incidence of the lymphocytic bronchitis and bronchiolitis compared to mice inhaling lower concentrations for one hour at a steady dose rate.

In the subchronic phase of the study, groups of mice were exposed to 0, 25, 50, and 100 ppm diacetyl, six hours a day, five days a week for 12 weeks. Mice exposed to 50 ppm and 100 ppm dose levels were found to have dose-dependant mild to moderate nasal tissue necrosis. Moderate lymphocytic inflammation of the bronchii with epithelial

atrophy was found in mice exposed to 100 ppm. Some minimal lymphocytic inflammation extended to the small airways in most of the high dose animals. In an effort to bypass the extensive removal of water-soluble diacetyl vapors that occurs in the nasal passages of mice, groups of animals were administered liquid diacetyl at dose levels of 100, 200, and 400 mg per kg body weight by oropharyngeal aspiration. This technique forces the test material deep into the lung. Two high-dose animals and one mid-dose animal died within two days of administration. By four days following aspiration, there were dose-dependent fibrotic foci in the terminal bronchioles and alveolar ducts of the remaining mice treated at 200 and 400 mg/kg diacetyl. Although these lesions were not identical to bronchiolitis obliterans, there was sufficient similarity to suspect that they may progress to bronchiolitis with continued exposure. The National Toxicology Program recently approved the nomination of BFV, diacetyl, and acetoin for longer-term inhalation testing for respiratory toxicity.

The above studies demonstrate that inhalation exposure to BFV can cause nasal and airway injury in rats and mice. However, in the NIOSH studies, rats that inhaled pure diacetyl vapors at air concentrations comparable to the diacetyl levels inhaled during the BFV exposures reproduced the epithelial necrosis in the nose and upper respiratory tissue but not the BFV-induced injury that occurred lower in the respiratory tract. This suggests the possibility that other undetermined and untested butter flavoring components might be contributing to the airway damage caused by rodent inhalation of the complex mixture.

Species differences in the pattern of respiratory tract lesions have yet to be fully explored. Although humans exposed to diacetyl-containing butter flavors experience nasal irritation and rhinitis, the primary target of toxicity is the bronchioles. These small airways are unaffected by diacetyl inhalation in rodents, although the pharyngeal aspiration experiments indicate that the chemical can damage these tissues if sufficient amounts of diacetyl reach the lungs. Penetration beyond the upper respiratory tract is likely influenced by the superior efficiency with which the highly developed rodent nasal cavity extracts water-soluble compounds, like diacetyl, from the airstream. However, human nasal turbinates are less able to remove diacetyl and humans, unlike rodents, can bypass nasal extraction altogether through mouth breathing. As a result, inhalation of

diacetyl in humans would be expected to achieve higher airway doses than rodents, and, therefore, deeper penetration into the respiratory tract. Since some butter flavors are produced as spray-dried powders, inhalation of small particulates containing encapsulated diacetyl and other potential airway-reactive constituents might further enhance respiratory tract penetration. Additional research may clarify the extent to which diacetyl and other butter flavoring chemicals reach and damage the lower airways following inhalation in rodents versus humans.

PART B

Prevalence Estimates

Selected prevalence and exposure data from the occupational investigations discussed in Part A were organized into four broad exposure categories as an initial step in evaluating the relationship between flavoring-related obstructive airway disease and exposure to the flavoring compound diacetyl. These exposure categories were as follows:

- High Task Exposure Category intended to include job operations with frequent short-term exposures to diacetyl concentration that average at or above 5 ppm.
- High Exposure Category intended to include job operations with exposures to full shift time-weighted average (TWA) diacetyl concentration equal to or above 0.5 ppm or job operations with average short-term exposures that range from 1 ppm but less than 5 ppm.
- Middle Exposure Category intended to include job operations with exposures to full-shift TWA diacetyl concentration at or above 0.05 ppm but less than 0.5 ppm and average short-term exposures that are less than 1 ppm.
- Low Exposure Category intended to include job operations with exposures to full-shift TWA diacetyl concentration below 0.05 ppm and average short-term exposures that are less than 1 ppm.

The health outcomes used in this initial categorization were airway obstruction and severe airway obstruction among groups of employees engaged in a specific job operation or who worked in a specific location. Since OSHA's data base consists of almost exclusively cross sectional investigations at a single point in time, prevalence estimates are presented (i.e. number of cases of airway obstruction cases in a population at one point in time) rather than the preferred incidence (i.e. number of cases of airway obstruction that will develop in a population over a specified period of time). The excess prevalence is the observed prevalence in excess of that expected. The exposure data were the average diacetyl air concentrations measured in the specific work environment. If possible, both full shift TWA, usually over an eight hour work period, and average short-term exposure levels were determined. The short-term exposure levels were generally measured or estimated over 5 to 30 minute periods of time. . The preliminary nature of these range estimations are consistent with the limited exposure data used to make the initial benefits predictions and assumptions used to estimate cost of airway obstruction avoided annually by the draft standard. OSHA intends to conduct a more thorough exposure – response analysis in the future as more data become available.

1. **Prevalence of Airway Obstruction**

Data from the NIOSH investigations of six microwave popcorn (MWP) plants conducted between 2000 and 2004 were used to derive prevalence estimates of airway obstruction for the exposure categories. Eight groups of exposure and prevalence from these investigations were viewed as informative for this purpose. Four of the involved groups of employees who had high diacetyl exposures that exceeded a full shift 0.5 ppm TWA and/or an average 1 ppm during short-term tasks. Prevalence estimates for the middle and low exposure categories were each based on these groups. While the observed prevalence was available for other subgroups of employees in the NIOSH microwave investigations, they were considered less informative for a variety of reasons such as inadequate or suspect exposure measurements, inadequately defined job duties, substantial overlap with populations already reflected in the groups cited here and very small sample size. In the NIOSH studies, airway obstruction cases were identified from lung spirometry tests where FEV_1 and FEV_1/FVC were below the lower limit of normal

using reference values for age, gender, height, and race from the third National Health and Nutrition Examination Survey (NHANES III). An adjusted background prevalence of airway obstruction (i.e. expected airway prevalence of an unexposed reference population of similar age, gender, smoking status, etc.) was not available for some data sets. In this circumstance, a background prevalence of 5.5 percent was utilized. This value is the expected prevalence for all production workers that underwent spirometry in the NIOSH aggregated study of the six microwave popcorn production facilities based on rates of airway obstruction reported from NHANES III after adjustment for age, race, gender, and smoking status. OSHA will continue to adjust background prevalence estimates for data sets from the NIOSH microwave popcorn investigations as additional data become available for future analyses.

Where possible, the full shift exposures were determined from the mean of the combined personal TWA measurements for diacetyl taken over the majority of the work shift. Geometric mean (GM) was preferentially used over arithmetic mean (AM) since it is less impacted by extreme values in a skewed distribution of data. In some instances where the data did not allow an estimation of geometric mean, it was necessary to rely on the reported arithmetic mean. Area monitoring for diacetyl only was done during the initial NIOSH survey of the Missouri microwave popcorn production plant in November, 2000. In order to estimate the personal exposures of diacetyl for employees in this survey, the November 2000 area monitoring was adjusted based on the ratio of personal and area exposures for diacetyl simultaneously determined in the plant at a later time.

Short-term exposure levels were estimated from real-time area monitoring in the breathing zone of workers performing routine high exposure tasks. Brief periods of elevated diacetyl were found during transfer of butter flavoring to heated mixing tanks and during handling freshly popped microwave popcorn as part of quality control operations. The average air concentration of diacetyl over the duration of the task was used to represent these short-term exposure levels. Since there is reason to suspect acetoin may also contribute to obstructive airway disease, acetoin concentration in selected data groups are also mentioned for informational purposes

<u>High Exposure Categories</u>

The four groups of data in the high exposure category consisted of MWP employees that worked in mixing rooms, in packaging areas in close proximity to improperly sealed mixing tanks, and in quality control rooms where large amounts of microwave popcorn were popped on a routine basis. The air monitoring and prevalence of airway obstruction from the data sets are summarized in Table 1. The production workers in data group #2 include the six quality control workers in data group #1 and a small number of mixers in data group #3. The excess prevalence ranged from 6 to 78 percent across the four data groups. This wide range of values applies to the subset of high task exposures (i.e. short-term air levels > 5ppm) as well as the rest of the high exposure category.

The low excess prevalence estimate of 6 percent comes from a large group of packaging area employees at three MWP production plants where the butter flavoring and popcorn kernels were combined in a microwavable bag. Mean personal full-shift diacetyl levels were found to be around 0.5 ppm in plants where the packaging lines were not adequately isolated from the tanks of butter flavoring.

Table 1: Air Monitoring and Prevalence of Airway Obstruction in the High Exposure Category

Exposure Category	Data Group	Population	Diacety (ppm) Full shift	task	Airway Obstruction Prevalence Observed	Background	Excess
High [includes high task] Exposure: TWA: ≥0.5 ppm OR Task: ≥1 ppm *Excess Prevalence Range*: 6.0 to 77.8 %	1	QC workers,11/ 2000 survey at MO MWP plant	0.4** (N=3)	4 (N=3)	83.3% (5/6)	5.5%	77.8%
	2	Production workers, 11/2000 survey at MO MWP plant	0.4-4.2** (N=37)	>1 (see text)	19.8% (19/96)	5.5%	14.3%
	3	Mixers >12 mo at 6 MWP plants	0.02-1.0*** (N=20)	5-40 (N=4)	19.2% (5/26)	5.5%	13.7%
	4	Non-isolated packagers at 3 MWP plant	0.5* (N=11)	0.5 (see text)	11.5% (14/122)	5.5%	6.0%

* geometric mean of personal samples ** geometric mean personal estimate from area samples *** arithmetic mean of personal samples

The high excess prevalence estimate of 78 percent comes from five out of six quality control workers with obstructed airways at a single plant where approximately 100 bags of popcorn were popped per eight hour work shift. While the full shift TWA diacetyl levels were generally lower than the packaging area employees just mentioned, the quality control workers were repeatedly exposed to high short-term exposure levels while pouring freshly popped corn into open containers. Workers from six MWP production plants with prolonged employment as mixers and production workers at the Missouri plant with high exposures to diacetyl had 14 percent excess occurrence of airway obstruction.

The prevalence of airway obstruction observed in these four groups was more than two-fold higher than the expected background prevalence. Several employees from these groups and former employees who worked the same job operations at the same plants had clinical findings consistent with bronchiolitis obliterans. This evidence strongly suggests that occupational exposures in this category are associated with an elevated risk of respiratory disease. The exposures and prevalence of airway obstruction for data groups 1 through 4 are discussed in more detail below.

Data Group #1. Five of six quality control workers at the Jasper Missouri MWP plant had airway obstruction in a November 2000 survey that took place prior to implementing engineering controls (NIOSH, 1/2006). The mean (GM) area TWA diacetyl in the quality control area was 0.5 ppm at the time of the survey. Subsequent industrial hygiene data showed that personal sampling generated an exposure level in the quality control location that was 0.84 times that of area sampling simultaneously monitored on the same work shifts (NIOSH, 8/2001). The estimated average personal full-shift TWA for diacetyl was 0.4 ppm after applying this adjustment factor. Real time peak breathing zone levels of diacetyl averaged 4-12 ppm while pouring heated popcorn into a container. This task resulted in approximately 4 ppm integrated over the five minute task. Since many bags of popcorn were cooked during the typical shift, it is likely that these short-term exposures were the major contributor to full shift air concentrations.

Data Group #2. NIOSH reported that 21 out of 116 employees at the Jasper Missouri

MWP plant had airway obstruction in the November 2000 survey (Kreiss, 2002). Twenty employees (two airway obstruction cases) did not work in the production area of the plant (see data group 7 described below). There were 19 airway obstruction cases from the remaining 96 microwave popcorn employees who worked as mixers, packagers, quality control, and maintenance employees. This was more than three times the age- and smoking-adjusted background prevalence for all plant workers. All but one of these airway obstruction cases did not respond to bronchodilators (i.e. fixed airway obstruction) and three cases suffered severe loss of pulmonary function (i.e. $FEV_1 < 40$ percent predicted). Five former mixers and four former packaging line operators from this plant were previously confirmed to have bronchiolitis obliterans by CT scan or lung biopsy.

The mean (GM) area TWA diacetyl ranged from 0.5 ppm in quality control to 26 ppm (AM = 38 ppm) in the mixing area at the time of the November 2000 survey (NIOSH, 1/2006). Air monitoring in January 2001, prior to installation of engineering controls, showed that personal sampling generated an exposure level in the mixing room that was 0.16 times that of area sampling simultaneously monitored on the same work shift (NIOSH, 8/2001). Based on these adjustments, the mean (GM) personal TWA for combined diacetyl is estimated to be 4.2 ppm (AM = 6.0 ppm) for mixing operators. As described in data group 1, the adjusted full-shift personal combined air levels were estimated to be 0.4 ppm for the quality control employees. Microwave popcorn packaging line operators represented about 80 percent of the production workers. The adjusted mean (GM) full shift personal TWA for diacetyl among packaging line operators was 1.1 ppm (AM = 1.5 ppm) estimated from area sampling (GM = 1.3 ppm) as described above.

Real time peak levels of diacetyl ranged from 4 to 17 ppm in the center of mixing room while butter flavoring was poured into a tank of heated soybean oil and the task resulted in approximately 5.5 ppm integrated over 30 to 60 minute duration. This short-term task-based sampling was done after improvements local exhaust and general room ventilation. The combined short-term breathing zone diacetyl levels in the mixing area were undoubtedly higher prior to implementation of these controls. Several hundred ppm of diacetyl were measured in the air space just above heated butter flavorings contained

in a holding tank. Real time monitoring showed that air levels of diacetyl varied minimally in the packaging area of the plant and estimated to average between 1 and 2 ppm.

Data Group #3. There were five cases of airway obstruction among 26 employees who worked as mixers for more than 12 months at the Jasper, Missouri and five other microwave popcorn plants (Kanwal et al., 2006). This was 4.3 times higher than the prevalence of airway obstruction among mixers with 12 months or less mixing experience (2 of 45 with obstructed airways) and 3.5 times the age- and smoking-adjusted background prevalence for the entire work force at all six plants. NIOSH reported that the observed prevalence was virtually the same if the Missouri MWP plant mixers were removed from the analysis. Four of the five mixers with more than 12 months experience had fixed airway obstruction that did not respond to bronchodilator treatment. The mean FEV_1 was 82 percent lower than predicted in the long-term mixers relative to the comparison groups (≥95 percent). Former mixers from four MWP plants (Jasper MO, Marion OH, Sioux City IA, and Phillips, NE) had CT scans and lung biopsies that confirmed bronchiolitis obliterans.

The mean (AM) personal TWA diacetyl was low (0.03 ppm) for the mixing operation at the Clearwater, Nebraska MWP plant where airway obstruction was not found among employees. Mixers also had low mean (AM) personal TWA for diacetyl (0.02 ppm) at the Iowa MWP plant. However, the industrial hygiene measurements were taken at the height of summer when relative humidity can be very high (NIOSH, 7/2004). There is considerable loss of diacetyl using the NIOSH analytical method when samples are collected under conditions of high humidity (e.g. 50-100% relative humidity). Therefore these diacetyl measurements may be greatly underestimated. Real time breathing zone measurements at the Iowa plant that were not affected by relative humidity showed that diacetyl concentrations averaged about 40 ppm over a ten minute period after pouring containers of liquid butter into the mixing tank. Several employees with mixing experience in this plant had evidence of airway obstruction (NIOSH, 12/2004). The mean (AM) personal TWA concentration for diacetyl measured at three MWP plants (Phillips NE, Marion OH, Ridgway IL) were 0.4 to 1.0 ppm . The estimated

mean personal TWA for diacetyl levels at the Missouri plant was higher (see data group #2 above). Real time measurements at the Ohio plant showed task oriented short-term levels of diacetyl that averaged 5 ppm over a 30 minute period following activities such as opening the lid to the mixing tanks or handling the butter flavoring mixture (NIOSH 12/2004). It is likely that these short-term high exposure tasks are major contributors to full shift air concentrations experienced by mixers at these microwave popcorn plants.

Data Group #4. There was an observed prevalence of 11.5 percent (14 of 122) for airway obstruction among packaging line workers who worked in close proximity to butter flavoring tanks at three MWP plants (Phillips NE; Ridgway IL, and Marion OH). This was 2.1 times greater than age- and smoking-adjusted background prevalence for the entire work force at all six plants. The observed prevalence of airway obstruction was also twice as high as that found among packaging workers in MWP plants where the tanks were isolated from the packaging area (Kanwal, 2006). Ninety percent of the cases that were administered bronchodilator were found to have fixed airway obstruction (i.e., 9 out of 10 tested) . Three airway obstructed packaging line workers from the Ridgway, Illinois plant had lung biopsies or CT scans consistent with bronchiolitis obliterans.

The mean (GM) personal TWA diacetyl (0.5 ppm) and acetoin (0.3 ppm) at the Ridgway Illinois plant (NIOSH, 10/2003) and the mean (GM) personal TWA diacetyl (0.4 ppm) and acetoin (0.6 ppm) at the Phillips, Nebraska plant (NIOSH, 5/2003) were considered to best represent the airborne exposures from packaging lines not adequately isolated from butter flavoring tanks. The mean (AM) personal TWA for diacetyl (0.02 ppm) among packaging operators at the Marion, Ohio plant were excluded, because these samples were taken only after ventilation improvements were implemented (NIOSH, 12/2004). NIOSH investigators considered these measurements to under-represent the packaging area exposures in this plant prior to the improvements in ventilation (Kanwal, 2006). The full shift TWA air concentrations in the microwave popcorn packaging areas are not strongly influenced by short-term, high exposure tasks as in the case of mixing and quality control areas.

Middle Exposure Category

There were two groups of exposure and prevalence data in the middle exposure category. One consisted of the prevalence of airway obstruction in employees that had ever worked in the quality control operation at the Ridgway, Illinois MWP plant (#5). Quality control operators at this plant cooked fewer bags of popcorn per shift on a less frequent basis than occurred at the Missouri plant (see data group #1). A second reflected packaging area employees hired after engineering controls had been implemented at the Jasper, Missouri MWP plant (#6). The corresponding air monitoring data, is summarized in Table 2. These groups of workers were exposed to TWA personal exposures to diacetyl that were between 0.05 and 0.5 ppm and routine short-term exposure levels that averaged less than 1 ppm, The excess prevalence of airway obstruction for data groups #4 and #5 were 10.2 and 1.1 percent, respectively.
In contrast to the high exposure category, the prevalence of airway obstruction in employees with exposures in the middle category was less than two-fold higher than the expected background prevalence and none of the employees were known to have developed clinical findings consistent with bronchiolitis obliterans. The evidence is less certain whether occupational exposures in this category are associated with an elevated risk of respiratory disease. OSHA is continuing to evaluate data on flavoring-related obstructive airway disease that might help further characterize the risk associated with exposures in this category. Data groups #5 and #6 are discussed in more detail below.

Table 2: Air Monitoring and Prevalence of Airway Obstruction in the Middle Exposure Category

Exposure Category	Data Group	Population	Diacetyl (ppm)		Airway Obstruction Prevalence		
			Full-shift	task	Observed	Background	Excess
Middle Exposure TWA: ≥0.05-0.5 ppm AND Task: <1 ppm *Excess Prevalence Range*: 1.1 to 10.2 %	5	QC Workers at IL MWP plant	0.06* (N=2)	0.25 (see text)	22.2% (2/9)	12.0% (3/25)	10.2%
	6	Packagers at MO MWP plant hired post-11/2000 survey	0.11* (N=91)	0.11 (see text)	6.6% (5/76)	5.5%	1.1%

* geometric mean of personal samples

Data Group #5. The observed prevalence of airway obstruction (22.2%) among

workers who had ever worked in quality control at the Ridgway, Illinois MWP plant was 1.6 times higher than that (12%) of workers who never worked quality control. The relatively large proportion of airway obstruction in both groups likely reflects, in part, exposures in production areas as well as quality control since many of the employees also worked as packaging line operators or mixers. For this reason, the 12 percent prevalence of workers who never worked quality control was considered a more appropriate internal background for estimating excess prevalence for this data group than the external adjusted NHANES III population. Mean (GM) personal full shift TWA exposure to diacetyl during quality control operations was 0.06 ppm (AM = 0.06 ppm) . Approximately 60 bags of microwave popcorn were popped over a ten hour shift by two quality control workers. Assuming that the exposure profile is similar to quality control operations at the Jasper, Missouri MWP plant, it is reasonable to expect that the vast majority of the full shift exposure for each worker occurred during a series of five minute operations that involved removing popped corn from microwave ovens and pouring the heated contents into a container. If it is further assumed that each quality control worker processes 30 bags of microwave popcorn per shift, the estimated diacetyl in the breathing zone would average 0.25 ppm over the five minute task.

Data Group #6. Seventy-six packaging area workers that were hired at the Jasper, Missouri MWP plant from December 2000 to April 2003 underwent multiple rounds of spirometry testing. This time period represents the post-implementation period during and after which engineering controls were put in place. The prevalence of airway obstruction among this cohort at initial spirometry was 5.3 percent and the prevalence at their last spirometry was 6.7%. The prevalence of airway obstruction at last spirometry was 1.3 times the prevalence at initial spirometry for this group. The initial spirometry usually occurred within three months of hire. The average length of employment between first and last spirometry was 12 months. Of the packaging area employees that had three or more rounds of spirometry, seven percent (3 of 41) had notable lung function interval declines (i.e. FEV_1 declines $\geq$300 ml or $\geq$ 10 percent decline in FEV_1 from one survey to the next). The mean (GM) personal TWA exposure to diacetyl in the packaging area between 2001 and 2003 was estimated to be 0.11 ppm based on four

industrial hygiene surveys (4/2001, 11/2001, 3/2002, 1/2003). Air sampling during summer months (9/2001, 8/2002) were not included in this mean TWA determination because of concerns about the performance of the NIOSH analytical method under conditions of high humidity. The highest mean TWA diacetyl concentration was 0.3 ppm in March 2002 and the lowest was below the limit of quantitation (0.003 ppm) in January 2003 after all new engineering controls were completely implemented. Real time air sampling indicated that diacetyl air levels varied minimally in the packaging area over a work shift relative to other microwave popcorn operations such as the mixing and quality control areas.

Low Exposure Category

There were two data groups in the low exposure category. One consisted of the prevalence of airway obstruction among MWP plant employees who worked in non-production areas (#7). A second reflected the prevalence of airway obstruction in MWP plant employees who worked in packaging areas that were isolated from mixing operations and tanks of butter flavoring (#8). The corresponding air monitoring data are summarized in Table 3. These groups of workers were exposed to mean personal TWA exposures of diacetyl of 0.05 ppm and below.

The excess prevalence of airway obstruction with exposures in the low category was generally smaller than the middle or high categories and included a reduced proportion of fixed obstruction (i.e. no improved pulmonary function with bronchodilator treatment). No excess prevalence of airway obstruction was found in data group #8 when compared to the expected background rate. Fifty percent of the cases were fixed obstruction. In data group #7, two out of 20 employees had airway obstruction (1.8-fold higher than background) but one employee did not have fixed obstruction and the other did not receive bronchodilator treatment. The proportion of fixed obstruction among those with flavoring-related obstructive lung disease is typically 80 to 90 percent (Kanwal et al., 2006). Data groups #7 and #8 are discussed in more detail below.

Table 3: Air Monitoring and Prevalence of Airway Obstruction in the Low Exposure Category

Exposure Category	Data Group	Population	Diacetyl (ppm) Full-shift	task	Airway Obstruction Prevalence Observed	Background	Excess
Low Exposure TWA: <0.05 ppm And Task < 1 ppm *Excess Prevalence Range*: 0 to 4.5 %	7	Non-production workers at MO MWP plant	0.02** (N=11)	0.02 (see text)	10.0% (2/20)	5.5%	4.5%
	8	Isolated packagers at two MWP plants	≤0.02*** (N=7)	≤0.02 (see text)	5.3% (4/75)	5.5%	0%

** geometric mean personal estimate from area samples *** arithmetic mean of personal samples

Data Group #7. Two of 20 employees who worked in non-production areas such as administrative offices, the warehouse, plain popcorn packaging, or outside at the Jasper, Missouri MWP plant had airway obstruction (NIOSH, 8/2001). This was 1.8 times the age- and smoking-adjusted background prevalence for all plant workers. The obstructed airways of one case were responsive to bronchodilator treatment. The other case did not receive bronchodilator administration due to possible health complications. Mean (GM) full shift area TWA for diacetyl was 0.01 ppm (AM = 0.04 ppm) for all non-production areas (NIOSH, 1/2006; 8/2001). Eight of the 11 measurements did not detect diacetyl but were assigned one half the limit of detection (i.e. 0.007 ppm). Concurrent monitoring of non-production areas in April 2001 indicated that personal sampling yielded, on average, 1.4-fold higher air levels than area sampling. The adjusted mean (GM) personal TWA for diacetyl is estimated to be 0.02 ppm. Short-term excursions of diacetyl above the TWA level in these job areas are not expected.

Data Group #8. There were four cases of airway obstruction out of 75 employees who worked in the packaging areas of two MWP plants (Sioux City, IA and Clearwater, NE). The packaging lines in these plants were located in a room isolated from the mixing tanks of butter flavoring (Kanwal et al., 2006). The observed prevalence was not elevated when compared to the age- and smoking-adjusted background prevalence for the entire work force at all six plants. Two of the four employees with obstructed airways were responsive to bronchodilator treatment. There were no known cases with clinical evidence of bronchiolitis obliterans among these employees. A very low mean (AM) personal TWA exposure of diacetyl of 0.003 was reported among packaging area workers

at the Iowa MWP plant where measurements were likely underestimated due to the effects of high relative humidity on the sampling method (NIOSH, 7/2004). The mean (AM) personal TWA diacetyl were 0.02 ppm for packagers at the Clearwater, Nebraska MWP plant (NIOSH, 7/2003). As mentioned in data group #4, personal sampling for diacetyl among packaging operators at another MWP plant (Marion, OH) was 0.03 ppm following ventilation and other improvements that effectively isolated the packaging lines from the butter flavoring mixing tanks.

2. **Severe Airway Obstruction**

Several occupational investigations identified employees with severe airway obstruction in plants where air levels of diacetyl were monitored in the workplace. These studies were used to derive prevalence estimates of severe airway obstruction across the exposure categories and to assist in predicting the benefits of preventing a serious and life-threatening respiratory condition. Severe airway obstruction usually refers an obstructive pattern with a FEV_1 that is 40 to 50 percent or less than predicted based on spirometry reference values. The background prevalence of severe airway obstruction is very low in the U.S. population, especially for young non-smokers. According to NHANES III, the prevalence of severe airway obstruction is about 0.1 percent for never-smokers and 0.6 percent for ever-smokers aged 17 to 49 years of age, and approximately 1.8 percent in older individuals 50 to 69 years of age. The expected background prevalence of severe airway obstruction for the data groups represented in the table range from 0.2 to 0.7 percent depending on the age and smoking status of the workers. For example, the adjusted prevalence was 0.5 percent for the production workers surveyed in the six microwave popcorn plants investigated by NIOSH.

There were four groups of employees who were regularly exposed to brief periods of diacetyl above 5 ppm during short-term tasks (i.e. high task exposure category). These tasks included pouring, mixing, blending and transferring pure diacetyl or flavorings containing diacetyl. Workers engaged in these operations appear to have the greatest prevalence of severe airway obstruction. An additional three sets of employees were used to estimate the prevalence range associated with full-shift TWA exposures to diacetyl above 0.5 ppm but lower short-term exposures than the high task exposure

category. No cases of severe airway obstruction were observed in the three groups in the middle and low categories where full shift TWA exposures were 0.5 ppm and below with average short-term air levels below 1 ppm.

High Task Exposure Category

The air monitoring and prevalence of severe airway obstruction for groups in the high task exposure category are summarized in Table 4. They involve separate groups of mixers at the Missouri and Ohio MWP plants, employees who formulate and blend powdered food flavorings at a California flavoring manufacturing plant, and process operators involved in the production of pure diacetyl at a chemical plant in the Netherlands.

Table 4: Air Monitoring and Prevalence of Severe Airway Obstruction in the High Task Exposure Category

Exposure Category	Data Group	Population	Diacetyl (ppm) Full-shift	Diacetyl (ppm) task	Airway Obstruction Prevalence Observed	Airway Obstruction Prevalence Background	Airway Obstruction Prevalence Excess
High Task Exposure Task: ≥ 5 ppm *Excess Prevalence Range* 2.2 to 37.5 %	9	Mixers at MO MWP plant pre- 11/2000 survey	4.2** (N=10)	>26 (see text)	38.0% (5/13)	0.5%	37.5%
	10	Mixers at OH MWP plant	1.0*** (N=7)	5 (N=4)	17.0% (2/12)	0.5%	16.5%
	11	Powder blenders at CA flavoring plant	0.05* (N=9)	>8* (N=2)	12.0% (3/25)	0.3%	11.7%
	12	Process workers at Dutch diacetyl plant	>2* (N=26)	>11* (N=4)	2.9% (3/102)	0.7%	2.2%

* geometric mean of personal samples ** geometric mean personal estimate from area samples *** arithmetic mean of personal samples

More than one-third of the employees who worked as mixers between 1992 and 2000 at the Missouri MWP plant developed bronchiolitis obliterans and severe loss of pulmonary function. These workers were routinely exposed to very high air concentrations of diacetyl while manually transferring butter flavorings to improperly sealed mixing tanks of heated soybean oil in a poorly ventilated work area. An observed 10 to 20 percent prevalence of severe airway obstruction occurred among two other groups of workers

who mixed or blended butter flavorings containing diacetyl. These employees encountered lower full shift exposures than the Missouri mixers but were, nevertheless, routinely exposed to high concentrations for brief periods while engaged in specific tasks. Three percent of plant employees that worked in chemical production of pure diacetyl were found to have severe airway obstruction and clinical signs of bronchiolitis obliterans. The occurrence of severe airway obstruction in these groups was 4 to 75 times the expected background prevalence. The exposures and prevalence of severe airway obstruction from each group are discussed in more detail below.

Data Group #9. Five former mixers from the Jasper, Missouri microwave popcorn plant were identified with severe fixed airway obstruction (FEV_1 18-35 percent of predicted) and clinical evidence consistent with bronchiolitis obliterans prior to the November 2000 NIOSH investigation (Akpinar-Elci, 2004). During the period between 1992 and 2000 when these individuals developed respiratory disease there were an estimated 13 employees that ever worked as mixers at the plant (MMWR, 2002). The observed prevalence of 38 percent is more 70 times the expected rate of severe airway obstruction. The mean (GM) area TWA diacetyl was 26 ppm (AM = 38 ppm) in the mixing area during the November 2000 survey (NIOSH, 1/2006). This exposure level adjusted to account for differences between area and personal sampling(see data group #2) gives an estimated mean (GM) personal TWA for diacetyl of 4.2 ppm (AM = 6.0 ppm). Since full shift area samples averaged 26 ppm, it is likely that short-term air levels in the breathing zone exceeded this during the manual transfer of butter flavoring into heated tanks of soybean oil. The estimated full shift and task-based air concentrations above are believed to be representative of the mixing area in prior years.

Data Group #10. Two of 12 mixing room workers at the Marion, Ohio MWP plant with fixed airway obstruction had serious loss of pulmonary function (i.e. FEV_1 approximately 50 percent of predicted). The observed prevalence was more than 30 times the expected rate (NIOSH, 12/2004). A former mixer at the plant was diagnosed with severe fixed obstructive airway disease consistent with bronchiolitis obliterans based on a CT scan. The mean (AM) personal TWA diacetyl concentration for mixers was 1.0 ppm. Real

time measurements showed task-oriented short-term levels of diacetyl that averaged roughly 5 ppm from activities such as opening the lid to the mixing tanks or handling the butter flavoring mixture. It is likely that these short-term high exposure tasks are major contributors to full shift air concentrations experienced by mixers at these microwave popcorn plants.

Data Group #11. Two former and one current production room worker from the Commerce, California flavor manufacturing plant had severe fixed airway obstruction from a total of less than 25 employees that had ever worked in the area over the entire history of the plant (NIOSH, 4/2007). The injured workers were never smokers under the age of 50 and had $FEV_1 < 35\%$ of the predicted value. The observed prevalence was more than 50 times the expected rate. The background prevalence was presumed to be slightly lower (i.e. 0.3 percent) than for the MWP plants due to the slightly younger median age (35 years) of the production workers and the higher proportion of never smokers (60 percent) employed at the flavoring plant. At least two of the three injured employees had a CT scan or lung biopsy that confirmed bronchiolitis obliterans. All injured workers were involved in the production of powdered butter and other flavorings containing diacetyl. The mean (GM) personal full shift TWA diacetyl and acetoin was 0.05 ppm (AM = 0.22 ppm) and 0.12 ppm (AM = 0.23) respectively, in the powder production room over five days of production. It was unclear whether flavorings containing these two chemicals were being manufactured on all five days of air sampling. Mean (GM) personal task-oriented diacetyl were 8 ppm (AM = 8 ppm) during a 70 to 100 minute simulated task of producing large batches of powdered butter and vanilla flavoring. Mean (GM) area measurements during the production task was 13 ppm (AM = 21 ppm) and real time measurements in the breathing zone found even higher peak levels during short-term tasks of pouring, blending, and packaging the powdered flavorings. It is likely that the short-term high exposure tasks are the major contributors to full shift air concentrations experienced by the formulators and blending operators who work in the powder production area of the plant.

Data Group #12. Three of 102 diacetyl process operators at a Dutch chemical plant were

identified with severe fixed obstructive airway disease ($FEV_1 \leq 42\%$ of predicted) consistent with bronchiolitis obliterans based on high resolution CT scans (Van Rooy, 2007). The median age of all workers at the plant that underwent spirometry was 51 years of age and one-third had never smoked. The age of the injured workers at onset of symptoms was 39 to 52 years and two were non-smokers. An additional case of bronchiolitis was uncovered later in a non-smoking worker who did not participate in the study. The observed prevalence was at least five times the background prevalence for a US reference population of similar age and smoking status (i.e. estimated 0.7 percent). The mean (GM) diacetyl levels from a limited amount of area breathing zone sampling during production averaged 2 ppm (AM = 8 ppm) over the last 8 of the 40 year study period. Mean task-based personal sampling during transfer of diacetyl into containers was 11 ppm (AM = 35 ppm).

High Exposure Category

The air monitoring and prevalence of severe airway obstruction for the three groups in the high exposure category are summarized in Table 5. All involved packaging line workers at MWP plants who worked in areas that were not isolated from the mixing tanks. Unlike the mixers and blenders in the previous category, they did not routinely perform high exposure tasks that resulted in average short-term air levels of diacetyl that exceeded 5 ppm. Their excess prevalence of severe airway obstruction ranged from 0.3 to 2.2 percent. Each data group is discussed in more detail below.

Table 5: Air Monitoring and Prevalence of Severe Airway Obstruction in the High Exposure Category

Exposure Category	Data Group	Population	Diacetyl (ppm)		Airway Obstruction Prevalence		
			Full-shift	task	Observed	Background	Excess
High Exposure TWA: ≥0.5 ppm or Task: 1 - 5 ppm	13	Packagers at MO MWP plant, 11/2000 survey	1.1** (N=22)	2 (see text)	2.7% (2/73)	0.5%	2.2%
Excess Prevalence Range 0.3 to 2.2 %	14	Packagers at MO MWP plant, pre-11/2000 survey	1.1** (N=22)	2 (see text)	1.5% (4/272)	0.5%	1.0%

	15	Non-isolated packagers at 3 other MWP plants	0.5* (N=11)	1 (see text)	0.8% (1/122)	0.5%	0.3%

* geometric mean of personal samples ** geometric mean personal estimate from area samples

Data Group #13. There was severe airway obstruction in two of 73 packaging area employees at the Missouri MWP plant in the November 2000 survey (NIOSH, 8/2001). This was about four times the adjusted background prevalence for all plant workers. The mean (GM) estimated full shift personal TWA diacetyl exposure was 1.1 ppm (AM = 1.5 ppm) as adjusted from area measurements of diacetyl in the packaging area (see data group #2). The full shift air concentrations in the microwave popcorn packaging area are not as strongly influenced by short-term, high exposure tasks as the mixing and quality control areas.

Data Group #14. Four former packaging area workers from the Missouri MWP plant were identified with severe fixed airway obstruction (FEV_1 15-40 percent of predicted) and clinical evidence consistent with bronchiolitis obliterans prior to the November 2000 NIOSH investigation (Akpinar-Elci, 2004). During the period between 1992 and 2000 when these individuals developed obstructive lung disease, there were an estimated 272 employees that ever worked as packagers at the plant (MMWR, 2002). The observed prevalence of 1.5 percent is about three times the expected rate of severe airway obstruction. The mean (GM) full shift personal TWA for diacetyl was estimated to be 1.1 ppm (AM = 1.5 ppm) in the packaging area during a November 2000 survey. This is believed to be representative of the packaging area in prior years.

Data Group #15. One of 122 employees who worked in the packaging areas at three microwave popcorn plants (Phillips NE Ridgway IL, and Marion OH) had severe airway obstruction. The packaging lines in the three MWP plants were located in close proximity to the tanks of butter flavoring.

The mean (GM) personal TWA diacetyl (0.5 ppm) and /acetoin (0.3 ppm) at the Illinois plant (NIOSH, 10/2003) and the mean (GM) personal TWA diacetyl (0.4 ppm)

and /acetoin (0.6 ppm) at the Phillips, Nebraska plant (NIOSH, 5/2003) were considered to best represent the airborne exposures from packaging lines not adequately isolated from butter flavoring tanks. The mean (AM) personal TWA for diacetyl (0.03 ppm) among packaging operators at the Marion, Ohio plant were excluded, because these samples were taken only after ventilation improvements were implemented (NIOSH, 12/2004). NIOSH investigators considered these measurements to under-represent the packaging area exposures in this plant prior to the improvements in ventilation (Kanwal, 2006). The full shift air concentrations in the microwave popcorn packaging area are not as strongly influenced by short-term, high exposure tasks as the mixing and quality control areas.

Middle and Low Exposure Categories

There was no observed severe airway obstruction three groups in the in the middle and low exposure categories as summarized in Table 6.

Table 6: Air Monitoring and Prevalence of Severe Airway Obstruction in the Middle and Low Exposure Categories

Exposure Category	Data Group	Population	Diacetyl (ppm) Full-shift	task	Airway Obstruction Prevalence Observed	Background	Excess
Mid Exposure TWA: ≥0.05-0.5 ppm Task: <1 ppm *Excess Prevalence Range*: 0 %	16	Packagers at MO MWP plant hired post-11/2000 survey	0.11* (N=91)	0.11 (see text)	0% (0/76)	0.5%	0%
Low Exposure TWA: <0.05 ppm Task: <1 ppm *Excess Prevalence Range*: 0 %	17	Non-production workers at MO MWP plant	0.02** (N=11)	0.02 (see text)	0% (0/20)	0.5%	0%
	18	Isolated packagers at 2 MWP plants	≤0.02*** (N=7)	≤0.02	0 % (0/75)	0.5%	0%

* geometric mean of personal samples ** geometric mean personal estimate from area samples *** arithmetic mean of personal samples

The middle exposure employees (#16) were hired as packagers after engineering controls

had been implemented at the Missouri MWP plant. The low exposure employees either worked in non-production areas (data group # 17) or worked in MWP packaging areas that were isolated from mixing operations and tanks of butter flavoring (data group #18). These groups were exposed to mean personal TWA diacetyllevels that were below 0.5 ppm and short-term levels below 1 ppm. Each data group is discussed in more detail below.

Data Group #16. Seventy-six packaging area workers hired at the Missouri MWP plant during and following the time period (12/2000 to 4/2003) when controls were being implemented underwent multiple rounds of spirometry testing. No cases of severe airway obstruction occurred among this cohort during the surveillance period. The average length of employment between first and last spirometry was 12 months. Of the packaging area employees that hat had three or more rounds of spirometry, seven percent (3 of 41) had notable lung function interval declines (i.e. FEV_1 declines $\geq$300 ml or $\geq$ 10 percent decline in FEV_1 from one survey to the next).

The mean (GM) personal TWA exposure to diacetyl in the packaging area between 2001 and 2003 was estimated to be 0.11 ppm based on four industrial hygiene surveys (4/2001, 11/2001, 3/2002, 1/2003). Air sampling during summer months (9/2001, 8/2002) were not included in this mean TWA determination because of concerns about the performance of the NIOSH analytical method under conditions of high humidity. The highest mean TWA diacetyl was 0.3 ppm in March 2002 and the lowest was below the limit of quantitation (0.003 ppm) in January 2003 after all new engineering controls were completely implemented. Real time air sampling indicated that diacetyl air levels varied minimally in the packaging area over a work shift relative to other microwave popcorn operations such as the mixing and quality control areas.

Data Group #17. None of the 20 employees who worked at the Missouri MWP plant in non production areas such as administrative offices, warehouse, plain popcorn packaging, or outside had severe airway obstruction. Mean (GM) full shift area TWA for diacetyl was 0.01 ppm (AM = 0.04 ppm) for all non-production areas (NIOSH, 1/2006; 10/2001). Eight of the 11 measurements did not detect diacetyl but were assigned one half the limit

of detection (i.e. 0.007 ppm). Using the personal to area monitoring adjustments described in data group #7 the mean (GM) personal TWA for diacetyl is estimated to be 0.02 ppm. Short-term excursions of combined diacetyl above the TWA level in these job areas are not expected.

Data Group #18. None of the 75 employees who worked in the packaging areas of two MWP plants (Sioux City, IA and Clearwater, NE) had severe airway obstruction. The packaging lines in these two microwave popcorn plants were located in a room separate from the mixing tanks of butter flavoring. A very low mean (AM) personal TWA exposure of diacetyl of 0.003 was reported among packaging area workers at the Iowa MWP plant where measurements were likely underestimated due to the effects of high relative humidity on the sampling method (NIOSH, 7/2004). The mean (AM) personal TWA diacetyl were 0.02 ppm for packagers at the Clearwater, Nebraska MWP plant (NIOSH, 7/2003). Personal sampling for diacetyl among packaging operators at another MWP plant (Marion, OH) was 0.03 ppm following ventilation and other improvements that effectively isolated the packaging lines from the butter flavoring mixing tanks. .The full shift air concentrations in the microwave popcorn packaging area are not as strongly influenced by short-term, high exposure tasks as the mixing and quality control areas.

3. Limitations and Uncertainties

This preliminary categorization is subject to several sources of uncertainty. Some of the health outcome data is derived from a relatively small number of employees. Small sample size can produce less stable prevalence estimates and limit the power to detect small elevations in prevalence of disease. Most of the studies were cross-sectional in design and did not include employees who may have developed flavoring-related obstructive airway disease and left the investigated facility. Because there is a lack of longitudinal studies, OSHA must rely on prevalence rather than the preferred incidence data as a measure of disease. Most of the exposure data are based on limited amount of air monitoring conducted over a few days that may not accurately reflect disease-related exposures that likely occurred over many months and years. The NIOSH method used to

determine air levels in many of the cited studies substantially underestimate diacetyl under conditions of high humidity. Efforts are underway to better understand the impact of this methodological flaw on the air monitoring collected during past NIOSH investigations and to develop an improved sampling collection method for future investigations. The initial assessment relates exposure intensity with prevalence of airway obstruction but does not account for length of time spent in exposure-related jobs (e.g., number of years worked) or the frequency of exposure (e.g. number of days a year exposure occurs). Finally, the current analysis is based on the prevalence of impairment at a given point in time and doesn't consider latency of disease or turnover in the workforce. OSHA plans to address these uncertainties and limitations as it continues to develop the risk assessment.

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